

# Macondo-1 well oil-derived polycyclic aromatic hydrocarbons in mesozooplankton from the northern Gulf of Mexico

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Received 7 October 2011; revised 7 December 2011; accepted 11 December 2011; published 14 January 2012.

[1] Mesozooplankton ( $>200\ \mu\text{m}$ ) collected in August and September of 2010 from the northern Gulf of Mexico show evidence of exposure to polycyclic aromatic hydrocarbons (PAHs). Multivariate statistical analysis revealed that distributions of PAHs extracted from mesozooplankton were related to the oil released from the ruptured British Petroleum Macondo-1 (M-1) well associated with the R/V *Deepwater Horizon* blowout. Mesozooplankton contained  $0.03\text{--}97.9\ \text{ng g}^{-1}$  of total PAHs and ratios of fluoranthene to fluoranthene + pyrene less than 0.44, indicating a liquid fossil fuel source. The distribution of PAHs isolated from mesozooplankton extracted in this study shows that the 2010 *Deepwater Horizon* spill may have contributed to contamination in the northern Gulf of Mexico ecosystem.

**Citation:** Mitra, S., et al. (2012), Macondo-1 well oil-derived polycyclic aromatic hydrocarbons in mesozooplankton from the northern Gulf of Mexico, *Geophys. Res. Lett.*, 39, L01605, doi:10.1029/2011GL049505.

## 1. Introduction

[2] An estimated 4.93 million barrels (1 barrel = 42 US gallons) of crude oil were released into the Northern Gulf of Mexico (nGOM) from the British Petroleum (BP) Macondo-1 (M-1) site (Federal Interagency Solutions Group, Oil budget calculator—*Deepwater Horizon*, 2010, [http://www.restorethegulf.gov/sites/default/files/documents/pdf/OilBudgetCalc\\_Full\\_HQ-Print\\_111110.pdf](http://www.restorethegulf.gov/sites/default/files/documents/pdf/OilBudgetCalc_Full_HQ-Print_111110.pdf)), the location of the R/V *Deepwater Horizon* (DWH) blowout. The total extent of the surface oil slick, derived from wind, ocean currents, aerial photography, and satellite imagery, was estimated to be 180,000 km (J. Amos, BP spill was greater

disaster than public knew, 2010, <http://mcabi.org/news/PR-Norse-Amos-2010.pdf>). Chemical evidence of subsurface oil from the leak was found as far away as 30 km south of the M-1 well [Diercks et al., 2010]. Oil which is a complex mixture of hydrocarbons and other chemicals, contains numerous PAHs [Connell, 1997; National Research Council, 2002]. These PAHs may be used as chemical fingerprints of specific types of oil released into natural environments [Blumer, 1976]. For example, PAH distributions, or relative abundances of low and high molecular weight PAHs, have been used to fingerprint oil and determine the provenance of various oil spills in the environment [Bennett et al., 2000; Stout et al., 2001; Christensen et al., 2004]. Despite extensive efforts at completely delineating the extent of the DWH oil spill, there have been no studies published to date addressing whether or not the spill introduced oil-derived toxic compounds into the pelagic food web of the nGOM.

[3] Mesozooplankton are useful sentinel organisms for oil-derived pollution [Carls et al., 2006] that serve as food for early life stages of fish and shrimp. Furthermore, they act as conduits for the movement of oil-derived contamination and other persistent organic pollutants through the marine food web [Clayton et al., 1977; Borgå et al., 2004; Sobek et al., 2006; Hallanger et al., 2011a, 2011c]. The objective of this study was to extract and analyze PAHs in mesozooplankton collected in the nGOM after the 2010 DWH spill. The null hypothesis of this study was that the relative PAH distributions in mesozooplankton collected throughout the Gulf of Mexico would not resemble the relative PAH distributions in oil collected from the M-1 well.

## 2. Methods

### 2.1. Mesozooplankton Sampling

[4] The mesozooplankton samples in this study were collected from within  $\sim 20\ \text{km}$  from the M-1 site and at distal stations grouped around 300 km south of the M-1 site (Figure 1). Mesozooplankton were sampled with a Multiple Open and Closing Net Environmental Sampling System (MOCNESS) [Wiebe et al., 1976]. The MOCNESS had a  $1\ \text{m}^2$  opening and a mesh size of  $200\ \mu\text{m}$ . Five to ten minute tows were conducted at the surface, and forty to sixty minute tows were conducted between 500 m and the surface in August and September of 2010 (auxiliary material, Table S1).<sup>1</sup> All mesozooplankton ( $>200\ \mu\text{m}$ ) were collected from the cod end of the nets and immediately frozen in glass vials.

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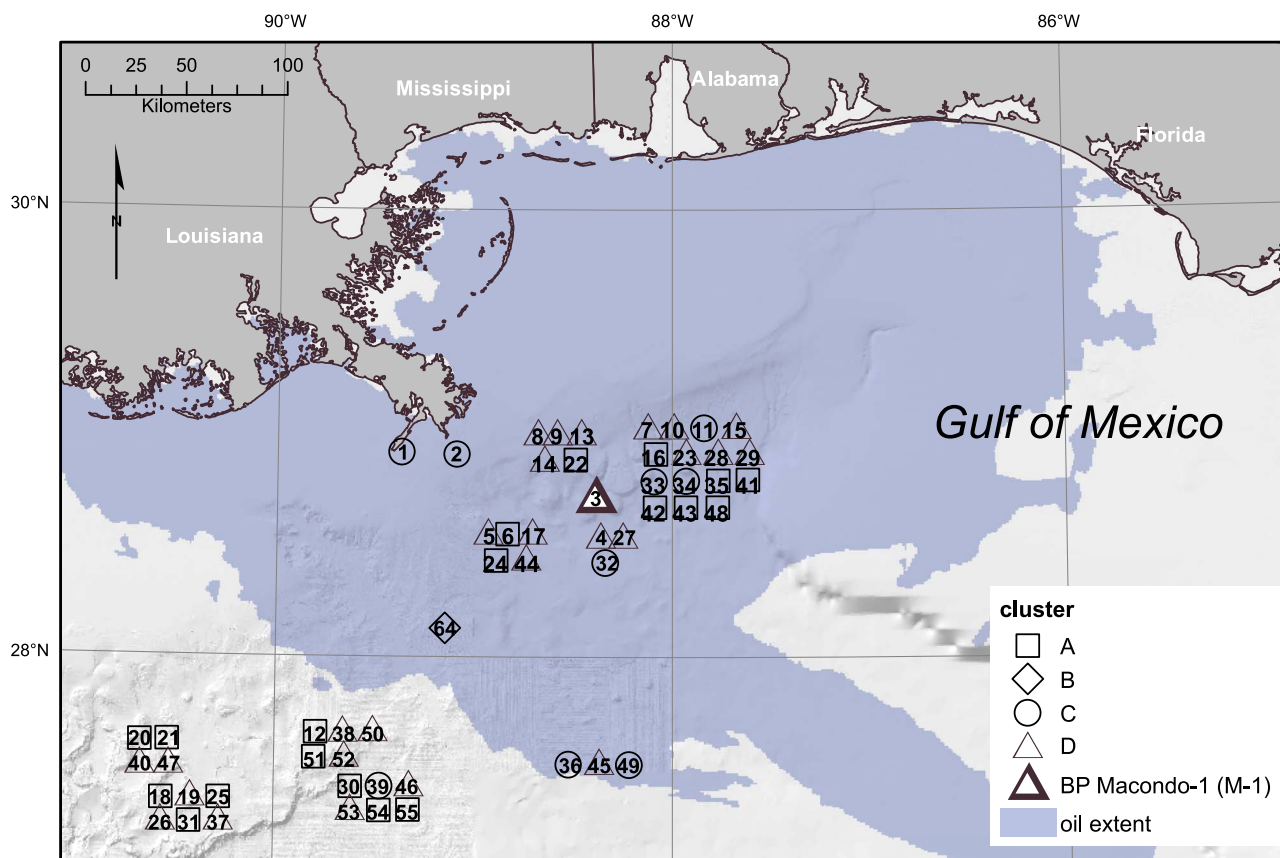
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**Figure 1.** Map of sampling area. Oil extent as of May 2010. Numbered symbols represent stations from which samples were analyzed for PAHs in this study.

This procedure generally resulted in two sets of samples from each station: one group of mesozooplankton samples from the surface water and one group integrated across the top 500 m water depth.

## 2.2. Oil, Surface Slick, and Seep Samples

[5] A sample of M-1 subsurface oil was provided by B & B Laboratory, College Station, Texas. The well oil was obtained by BP from the riser insertion tube aboard the drillship *Discoverer Enterprise* on May 21, 2010, and was absent of any defoamer or dispersant. All samples were collected, processed, and shipped under standard chain-of-custody protocols according to methods listed in the USGS National Field Manual for the Collection of Water-Quality Data (NFM) (<http://pubs.water.usgs.gov/twri9A/>) as well as other USGS standard operation procedures [Wilde *et al.*, 2010]. Surface slick samples were obtained manually in pre-cleaned I-Chem jars on May 8. The only available natural seep samples from the Gulf of Mexico were collected in 2002. These had been stored frozen at  $-20^{\circ}\text{C}$  at the USGS Menlo Park. Santa Barbara Channel samples, which consisted of sludge, seep oil, and produced oil, were collected in 2001, 2005, and 2008 and frozen until they were extracted for PAHs in this study.

## 2.3. Polycyclic Aromatic Hydrocarbon (PAH) Extraction

[6] Less than a gram of each oil or seep sample was weighed out in pre-cleaned ( $450^{\circ}\text{C}$  for 4h) aluminum foil

tins and transferred to vials to be extracted. Following determination of the sample weight, two mL of deuterated PAH surrogate standard (in acetone) were added to each oil, zooplankton, and seep sample. Then, zooplankton samples were macerated in a Sentry tissue macerator after which PAHs were extracted from them using an Accelerated Solvent Extractor (ASE) or sonication using hexane: acetone (1:1, v:v). All extracts were concentrated by rotary-evaporation followed by a  $\text{N}_2$  stream. Extracts were purified via silica gel and sodium sulfate chromatography on an open column. The aromatic fraction was eluted with 75 mL of 80:20 hexane:methylene chloride. Zooplankton PAH concentrations are reported on a wet weight basis. Additional details of sample collection and extractions, including extraction recoveries, may be found in the auxiliary material.

## 2.4. Data Analysis

[7] Data analyses were conducted using R statistical software version 2.10.1 (©R Foundation for Statistical Computing, 2009). The proportion of each of the 24 PAHs was calculated in each mesozooplankton extract by dividing individual PAH concentrations by the total concentration. We created a data matrix of columns containing proportional concentrations of each PAH compound and rows representing individual samples. Relationships between the PAH distributions in mesozooplankton and oil samples were characterized using a two-stage multivariate analysis. First, we performed an agglomerative, hierarchical cluster analysis

of a matrix of distances calculated using the `dist.prop` function in the R library `ade4` [Dray and Dufour, 2007], we calculated the distances between each row based on the suite of PAH compounds. The cluster analysis was conducted using the `hclust` function using Ward's linkage. To identify particular groupings of data, we used the resultant cluster analysis fit and cut the tree into 4 distinct groups using the `cutree` function. Using the resulting 4 groupings from this analysis, we calculated the average ( $\pm$  SD) proportion of each PAH within each group. A non-metric multidimensional scaling (NMDS) analysis was performed on the matrix of calculated distances using the `isoMDS` function in the R library `MASS` [Venables and Ripley, 1999].

### 3. Results and Discussion

[8] There were no systematic trends in PAH distributions in nGOM mesozooplankton as a function of radial distance from the M-1 well or with depth in the water column from which the organisms were collected. The lack of any difference in PAH distributions in mesozooplankton collected from surface or deeper (0–500 m) waters is not surprising given that many species of mesozooplankton exhibit diel vertical migration patterns [Haney, 1988]. In contrast to oil spills occurring at the sea surface, petroleum hydrocarbons originating from the M-1 well were subjected to several unique environmental processes that may account for the unique PAH distributions within each cluster group. First, petroleum was released from the Macondo well at 1.5-km depth. This resulted in partitioning of hydrocarbons into the aqueous phase in the absence of atmospheric evaporation [Reddy *et al.*, 2011]. Furthermore, the dispersants applied to this spill may have enhanced aqueous dissolution of oil droplets, affecting overall water column concentrations of PAHs in a manner in which there was no systematic geographical pattern with distance from the M-1 well.

[9] Cluster analysis suggests that PAH distributions in all samples analyzed could be divided into four distinct groups (Figure 2, top). Group A corresponded to mesozooplankton that were dissimilar to any oil samples analyzed in this study. The PAHs that were detected in natural seeps in the Gulf of Mexico (Mississippi Canyon, Figure 1, Station 64) clustered in Group B along with PAH distributions in produced oil, crude oil and tar balls from several locations in the Santa Barbara Channel. The distributions of PAHs in mesozooplankton from the other stations led to their emplacement in Groups C and D. Distributions of PAHs in Groups C and D were similar to PAH distributions in from the DWH surface slick and from the broken M-1 riser pipe, respectively.

[10] We used a non-parametric multidimensional scaling analysis on the distance matrix calculated for the cluster

analysis [Cox and Cox, 1994] to help visualize relationships in PAH distributions between mesozooplankton, DWH oil, and seep samples (Figure 2, bottom). For a majority of the mesozooplankton samples, PAHs detected in the nGOM mesozooplankton scaled closely to either Cluster C (PAHs in the DWH surface slicks) or Cluster D (PAHs in oil from the M-1 riser pipe). The PAHs in the nGOM seep and SBC samples clustered together, but did not scale closely to DWH oil-derived PAH distributions (Cluster B). Our results indicate that oil derived from the DWH incident was associated with mesozooplankton collected as far as 180 km from the M-1 well. Zooplankton in Cluster A do not have a PAH signature associated with DWH riser oil or DWH-derived surface slicks. This observation suggests that the oil release from the DWH incident, although spatially-extensive, may have been patchy or that these zooplankton were exposed to oil, but no longer have a relative PAH distribution that matches that of the DWH oil.

[11] The PAH distributions detected in some nGOM mesozooplankton in this study match the PAH distributions extracted in oil released from the broken riser pipe collected from the M-1 well and from surface oil slicks originating from the DWH incident (Figure 3). A two-sample Kolmogorov-Smirnov goodness-of-fit (KS-GOF) test comparing the mean PAH distributions revealed no differences in PAH signatures between Cluster B and C ( $p = 0.68$ ), Cluster B and D ( $p = 0.45$ ), and Cluster C and D ( $p = 0.26$ ). Mesozooplankton PAH signatures from Cluster A (Figure 3a), although relatively abundant in naphthalene, did not possess the ratios of fluorene:phenanthrene resembling those in the DWH surface slicks or oil from the broken riser pipe (Figures 3b and 3c). Comparisons of these PAH distributions showed significant differences when compared with the KS-GOF, Cluster A and B ( $p = 0.03$ ), Cluster A and C ( $p = 0.004$ ), and Cluster A and D were not statistically different at  $\alpha = 0.05$ , but did have a low  $p$ -value of 0.07. The PAH distributions from the SBC and the nGOM seep sample had much higher proportions of 1-methyl and 2-methylnaphthalene (Figure 3b) than the M-1 samples or the nGOM surface slicks (Figures 3c and 3d). The dissimilarity in these particular PAHs between leaking oil from the M-1 well and the natural GOM seep samples can be explained by chemical heterogeneity resulting from varying oil source rock, age, and migration history that likely occurs between the seep location and the M-1 site [Aharon *et al.*, 1997; Hood *et al.*, 2002].

[12] We are not aware of any existing studies examining PAH distributions in zooplankton collected from the water column of the nGOM, therefore we have no means of comparing PAH body burdens in nGOM zooplankton collected prior to the spill. Distributions of PAHs in fluvial suspended sediments such as those exported from the Mississippi River generally contain elevated relative

**Figure 2.** (top) Agglomerative, hierarchical cluster analysis of PAH distributions in oil from the broken riser pipe at the M-1 well (DWH.D), *Deepwater Horizon* surface slick oil (DWH.S), mesozooplankton collected from surface tows (ZP.S) and tows from 0–500 m water depth in nGOM (ZP.D). A natural seep sample from the nGOM (GMS) and oil samples derived from the Santa Barbara channel (SBC). The four letters in both plots represent the 4 groups identified in the cluster analysis (see text for explanations). (bottom) Non-metric multi-dimensional scaling (NMDS) biplot of PAH distributions in oil from the broken riser pipe at the M-1 well (3, oval), *Deepwater Horizon* surface slick oil (1,2, circles), nGOM seep samples (64, squares) and oil samples derived from a variety of locations in the Santa Barbara channel (56–63, hexagons). Other numbers are representative of mesozooplankton samples (see Figure 1 for collection location). The stress of the NMDS fit was 0.14. The principal variables structuring the data are the PAH signatures within each group.

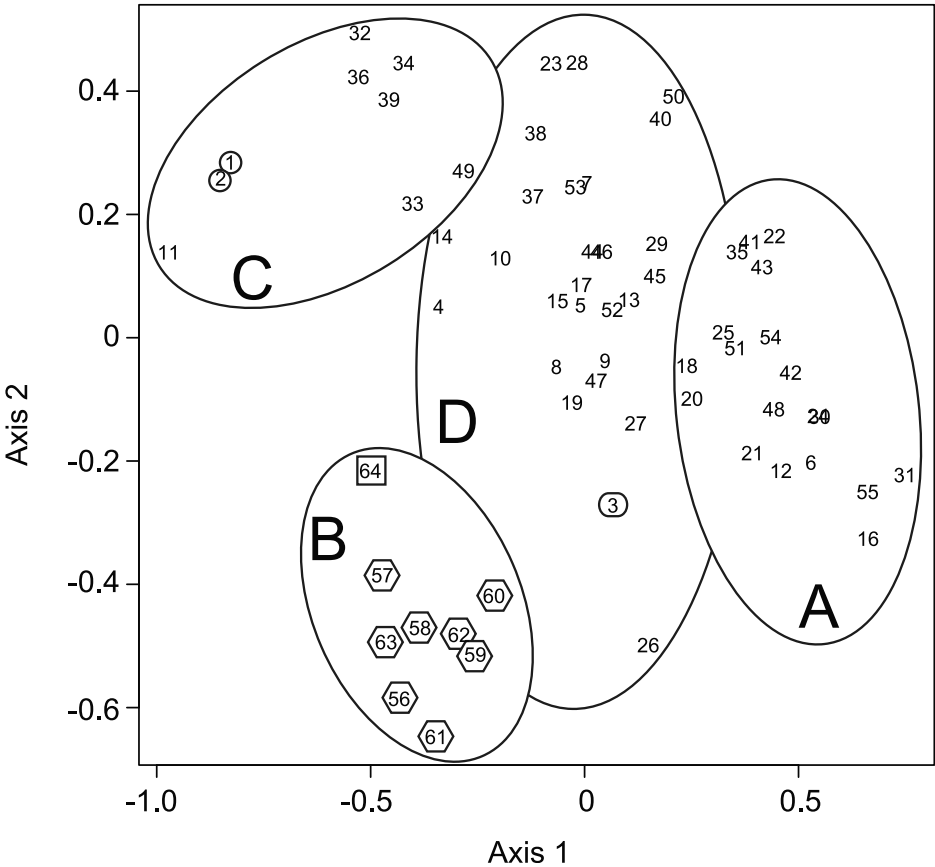
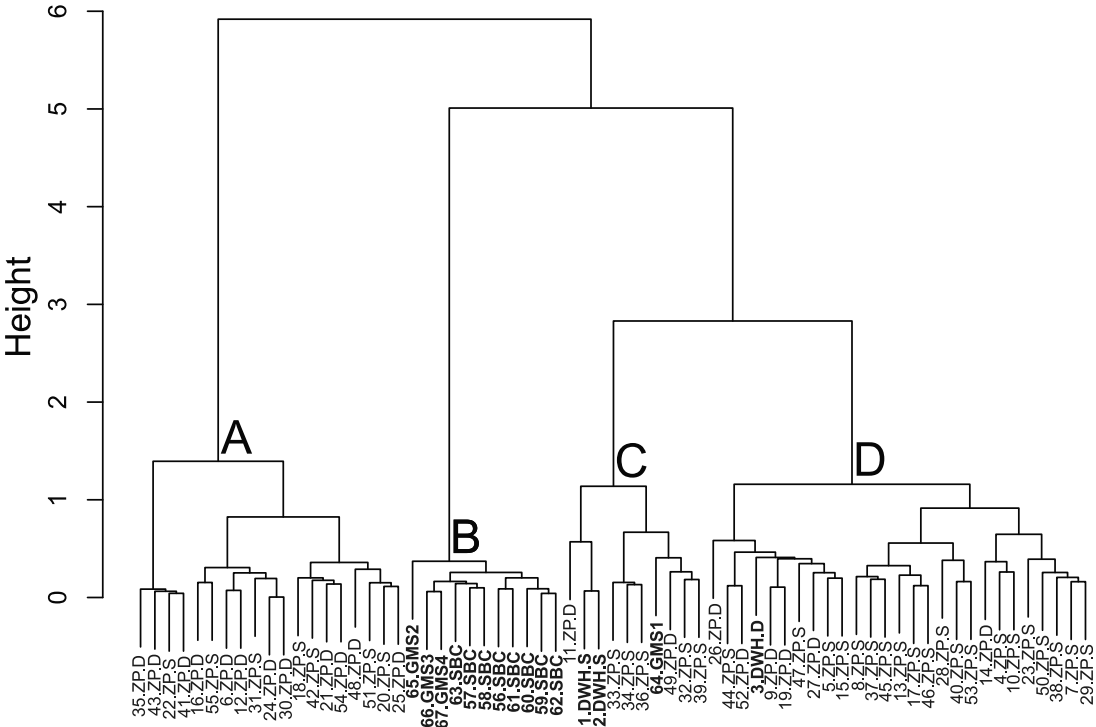
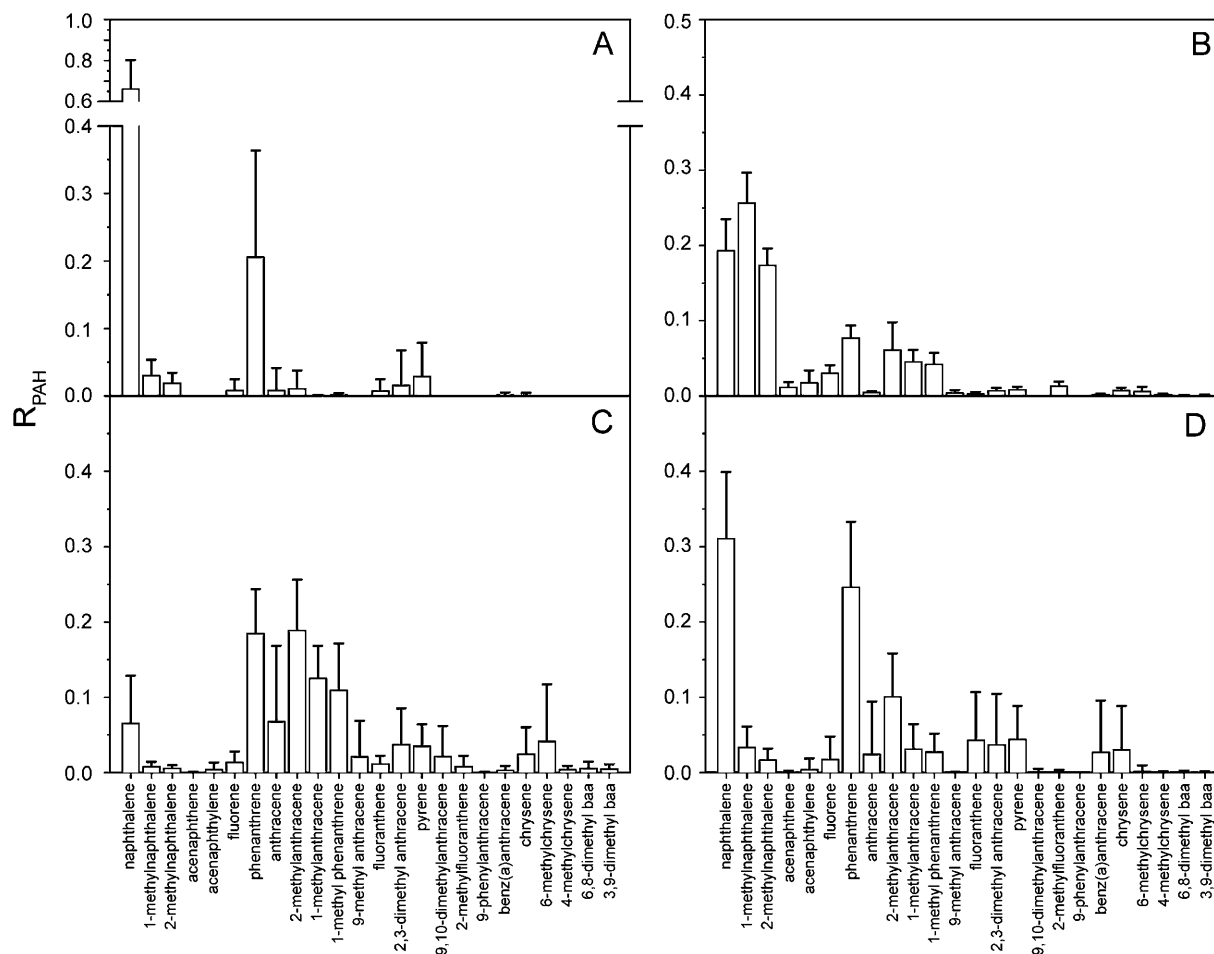


Figure 2



**Figure 3.** Mean ratio of individual PAHs to total PAHs ( $R$ ) within groups identified using the cluster analysis. (a) Mesozooplankton samples only, (b) Gulf of Mexico seeps and Santa Barbara Channel, (c) mesozooplankton samples and surface slick oil, and (d) mesozooplankton samples and oil from the leaking riser pipe.

abundances of higher molecular weight PAHs [Mitra *et al.*, 2002] than found in mesozooplankton analyzed in this study. Thermodynamic modeling of PAH distributions on particles has demonstrated that PAHs are strongly-sorbed onto Mississippi River and Gulf of Mexico sediments, which contain a non-trivial fraction of combustion-derived black carbon [Mitra and Bianchi, 2003]. Furthermore, ratios of fluoranthene:pyrene in these mesozooplankton samples are less than 0.5 (Table 1), as is expected in PAH distributions originating from a petrogenic source [Yunker *et al.*, 2002]. Taken together, this suggests that PAHs from suspended sediments did not contribute to the PAH signal detected in these

mesozooplankton samples and that the PAH distributions in these samples are petrogenic.

[13] There are numerous factors that may affect PAH body burdens in zooplankton isolated for this study. Unlike the elevated levels of PAHs accumulated in mesozooplankton collected in Port Valdez, Alaska [Carls *et al.*, 2006], the mesozooplankton PAH concentrations in our study (Table 1) are similar to that found in zooplankton collected near other oil spills in temperate and tropical environments globally [Davenport, 1982; Guzman del Proo *et al.*, 1986]. Several processes (e.g. exchange with water through passive partitioning, ingestion of contaminated food, and production of

**Table 1.** Table of PAH Concentrations in Each Hierarchical Cluster Group<sup>a</sup>

Cluster	Sample Type	N	Mean	Standard Deviation	Minimum	Maximum	flu/flu + pyr
A	mesozooplankton	19	17.4	28.9	1.64	120.0	0.19 ± 0.23
B	Santa Barbara Channel seep samples	8	1.23E + 06	6.91E + 05	4.96E + 05	2.61E + 06	0.25 ± 0.14
	nGOM seeps	4	1.19E + 04	1.15E + 04	1.60E + 03	2.70E + 04	0.29 ± 0.11
C	mesozooplankton	7	29.0	26.1	11.1	85.7	0.17 ± 0.22
	surface slicks	2	5.45E + 05		5.40E + 05	5.439 + 05	0.41
D	mesozooplankton	26	13.8	19.7	2.04	97.9	0.44 ± 0.29
	M-1 well	1	3.61E + 06		3.61E + 06	3.61E + 06	0.44

<sup>a</sup>Concentrations are given in ng g<sup>-1</sup> wet weight. N signifies number of samples extracted from within that group.

fecal pellets and eggs), occurring simultaneously, may affect the final body burden of PAHs derived from oil spills [Sobek *et al.*, 2006; Berrojalbiz *et al.*, 2011]. Moreover, all of these processes may vary as a function of ambient temperature and seasonality [Hallanger *et al.*, 2011b].

[14] Mesozooplankton species in microcosm experiments have been shown to excrete PAHs on time scales of days [Berrojalbiz *et al.*, 2009]. However, we detected the presence of PAHs in mesozooplankton collected in August and early September 2010, well after the M-1 well was capped on 15 July, 2010. This was surprising given that mesozooplankton population turnover times may be quite rapid in the warmer waters of the Gulf of Mexico (e.g. *Acartia tonsa* has a generation time of 7 days at 25 °C [Heinle, 1966]). We offer several possible explanations for the persistence of a low but DWH-derived PAH signal so long after capping of the well. First, the mesozooplankton samples in this study consisted of several species homogenized together; thus, cross-species metabolism of PAHs may vary resulting in relatively lower body burdens than seen in many single species laboratory studies. Second, PAHs from DWH oil may have remained in the system at significant levels long after the well was capped. Lastly, mesozooplankton may have been accumulating PAHs in their bodies and passing them across generations via eggs, which are relatively lipid rich compared to individual mesozooplankton. Determining which of these explanations is responsible for the ng g<sup>-1</sup> observed levels of PAHs in nGOM mesozooplankton is beyond the scope of this study. However, our study demonstrates that there was a signature distribution of DWH-derived PAHs in zooplankton.

[15] As of August 2010, the U.S. National Incident Command Center estimated that ~26% of the residual oil could be found either on or below the surface as light sheen and weathered tar balls, washed ashore or collected from the shore, or buried in sand and sediments (Federal Interagency Solutions Group, Oil budget calculator, 2010). Although a subsurface oil plume was identified [Camilli *et al.*, 2010] and its composition recently elucidated [Reddy *et al.*, 2012], the ultimate fate of the oil and its presence in the ecosystem has yet to be comprehensively determined. The presence of this PAH signature in nGOM mesozooplankton samples in the patterns noted in our study suggests that the spatial and temporal extent of the 2010 spill in the nGOM was extensive and patchy. A recent study reported a depleted  $\delta^{13}\text{C}$  isotopic signature in coastal mesozooplankton collected north of the M-1 well showing that carbon in oil collected at depth was incorporated up to two trophic levels above prokaryotic hydrocarbon consumers and into the planktonic food web [Graham *et al.*, 2010]. That study, combined with ours, suggests that the potential for movement of DWH-derived carbon and PAHs to higher trophic levels, existed after the well at M-1 had been capped.

[16] **Acknowledgments.** The authors thank the National Science Foundation RAPID grants OCE-1043249, OCE-1047736, OCE-1057461, and the captain and crew of the *R/V Oceanus*.

[17] The Editor thanks two anonymous reviewers for their assistance in evaluating this paper.

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